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Some Observations on the Seed Coat Structure within the Genus *Epilobium*

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Some Observations on the Seed Coat Structure within the Genus *Epilobium*

The genus *Epilobium* is a very variable one whose species exhibit a wide range of morphological flexibility¹. It is not surprising then to read in the literature that there may well be over 200 species throughout the world within this genus¹⁻². Furthermore the complexity of the genus has led to much difficulty in determining the distribution patterns of the various taxa, simply because of the lack of knowledge of the total variability within species³.

Despite the variable nature of the genus, autoploidy, rather than allopolyploidy, seems to occur in such variable species as *E. latifolium* and *E. angustifolium*^{4,5}. Cytotaxonomically the general chromosome number is $n = 18$, exceptions in North America do occur with such counts as $n = 11, 12, 13$ and 15^6 . Such variability in chromosome number (and inevitably ploidy) seems to be restricted to North Western America, specifically Nevada and the northern coastal regions of California.

Distributionally the genus is quite cosmopolitan and many species are circumboreal. Ecologically there seems to be little to indicate habitat specificity (many are weeds) with the possible exception of *E. latifolium* which appears to grow in abundance on gravel bars, although it is also found in sup-alpine regions, and *E. hornemannii* which often is found in flowing waterlogged areas.

One area of investigation which seems to have been overlooked is the constancy of the seed coat characteristics. Several workers have suggested that seed coat structure may well be of value in rather otherwise variable groups, because of the constancy of this character⁷. Indeed within the genus *Epilobium* seed coat sculpturing is used for certain species identification (papillate vs. smooth).

In this study, the seed coat structure of 17 North American taxa within the genus have been investigated using light stereo-microscopy, scanning electron microscopy and seed coat sectioning, to ascertain more critically the value of seed coat structure within the genus.

The seeds of the taxa under investigation were obtained both from the field and the University of Alberta Herbarium. They were fixed for 12 h at 0°C in 3% glutaraldehyde in 0.025 M phosphate buffer pH 6.9. After several buffer series the seeds were post fixed for 3 h in 2% buffered osmium tetroxide at 0°C. Dehydration was carried out with a graded series of ethanol. The tissues were embedded in SPURR's resin⁸.

Thick sections, 1–1.5 μm , were cut with a glass knife on a Reichert OMU₂ ultra-microtome and collected on gelatin coated glass slides⁹. Periodic acid-Schiff's¹⁰ and aniline blue black¹¹ procedures were used in order to demonstrate the morphology of the seed coat. Photographs

were obtained with a Zeiss photomicroscope using Kodak Plus X Pan film.

Electron micrographs of the cell wall structure (Figure 12) was obtained from ultra thin sections cut from the same blocks used for light microscopy. The sections were stained sequentially with aqueous uranyl acetate and lead citrate¹², and viewed under a Phillips 200 E.M. Seeds from the same source were glued to E.M. stubs and coated sequentially with 100 Å thick layer of carbon and gold and viewed with a Cambridge stereoscan S4.

Keys to Figures 1–12

Seed coat structure	Species	Figures
Papillate	<i>E. glandulosum</i>	1, 7
	<i>E. platyphyllum</i> Rydb.	2, 8, 12
	<i>E. hornemannii</i> Reichenb.	2, 8, 12
	<i>E. clavatum</i> Trel.	2, 8, 12
Sub-papillate	<i>E. paniculatum</i> Nutt.	3, 9
	<i>E. hirsutum</i>	3, 9
	<i>E. anagallidifolium</i> Lam.	3, 9
	<i>E. davuricum</i> Fisch.	4, 8
	<i>E. leptophyllum</i> Raf.	4, 8
	<i>E. palustre</i> L.	4, 8
	<i>E. palustre</i> var. <i>grammadophyllum</i>	4, 8
Smooth	<i>E. palustre</i> var. <i>monticola</i>	4, 8
	<i>E. angustifolium</i> L.	6, 11
Foveolate	<i>E. latifolium</i> L.	6, 11
	<i>E. lactiflorum</i> Hausskn.	5, 10
	<i>E. alpinum</i> var. <i>alpinum</i>	5, 10
	<i>E. luteum</i> Pursh	5, 10

¹ P. A. MUNZ, in *North American Flora II* (W. H. Scripps, Claremont, California, USA 1965), part 5, p. 1–231.

² P. H. RAVEN, Bull. Br. Mus. Nat. Hist. Bot. 2, 325 (1962).

³ E. HULTÉN, *Flora of Alaska and Neighbouring Territories* (Stanford University Press, California 1968).

⁴ T. MOSQUIN, Brittonia 18, 167 (1966).

⁵ E. SMALL, Brittonia 20, 169 (1968).

⁶ H. LEWIS and P. H. RAVEN, *Records Advanced Botany* (University of Toronto Press, Toronto 1961), vol. 2, p. 1466.

⁷ P. H. DAVIS and V. H. HEYWOOD, *Principles of Angiosperm Taxonomy* (Olivier and Boyd, Edinburgh and London 1963).

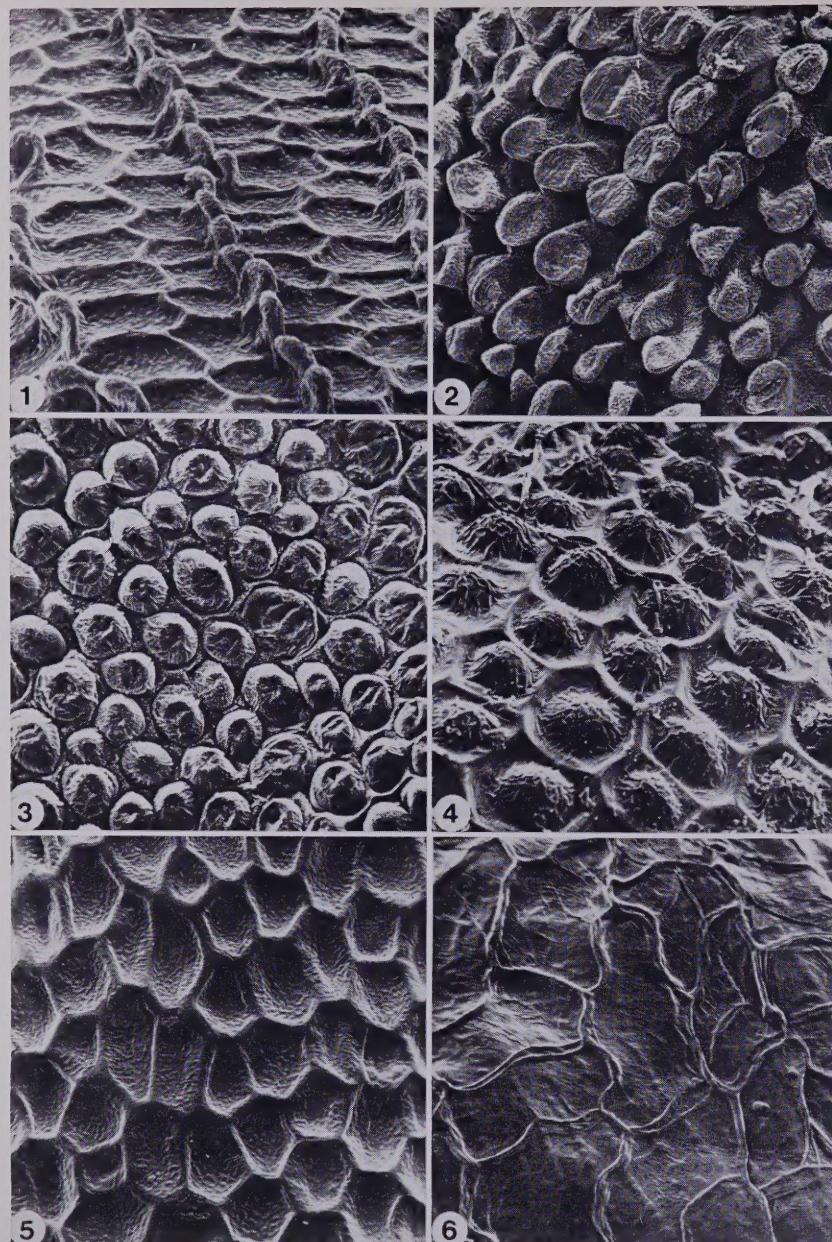
⁸ A. R. SPURR, J. Ultrastruct. Res. 26, 31 (1969).

⁹ W. A. JENSEN, *Botanical Histochemistry* (W. H. FREEMAN & CO, San Francisco 1962).

¹⁰ R. D. HOTCHKISS, Arch. Biochem. 16, 131 (1948).

¹¹ D. B. FISHER, Histochemie 16, 92 (1968).

¹² E. S. REYNOLDS, J. Cell Biol. 17, 208 (1963).



Figs. 1-6. S.E.M. seed coat ornamentation. $\times 900$.

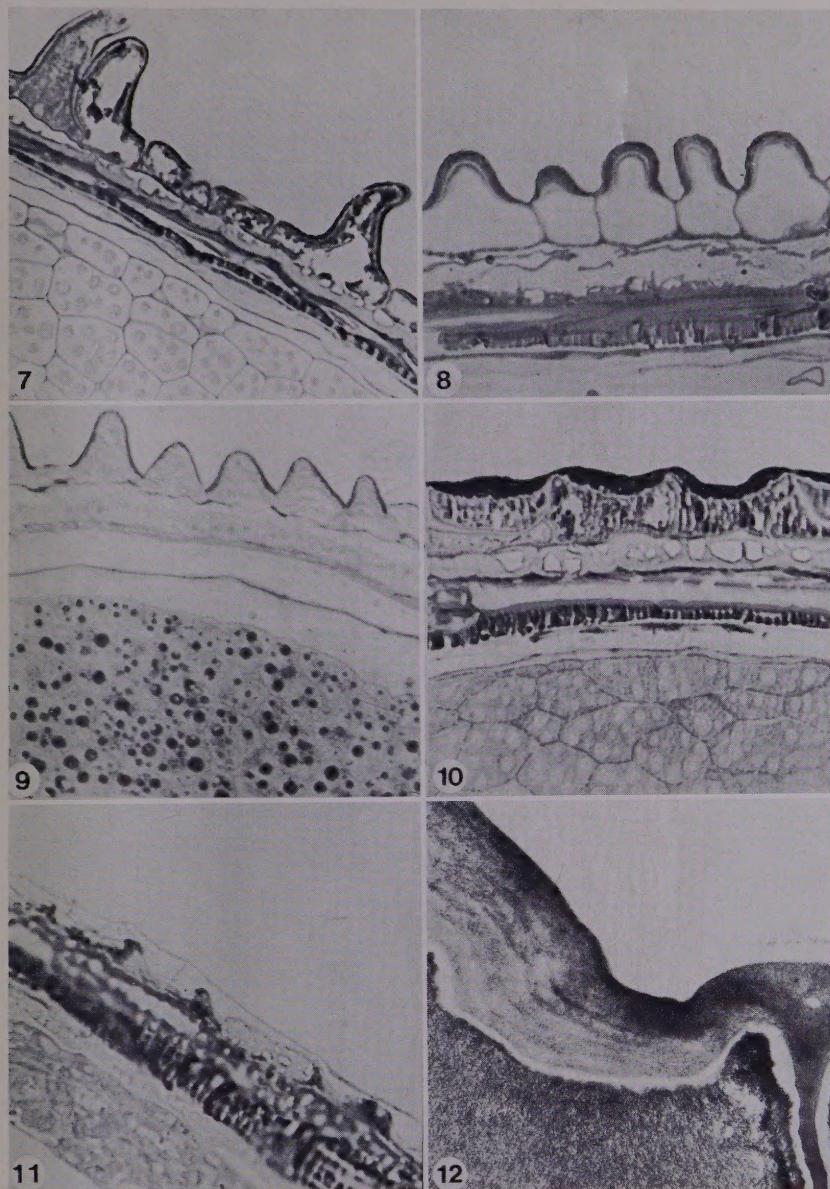
Under both the light microscope and S.E.M., it was very apparent that the taxa investigated fall into 2 groups with respect to their seed coat structure, papillate and smooth. However, within both of these categories it is apparent also that papillation is of 2 types, as is the nature of the so called smooth seeded group.

The 2 types of papillae present are illustrated in Figures 2 and 4. In one the whole of the epidermal cell appears to be modified into a papilla and is substantially thickened on its outer wall. This is noticeable under light microscope sections Figure 8 and also the layered structure is clearly visible under transmission E.M. (Figure 12). The other type of papillation, for convenience referred to as sub-papillate, involves only part of the cell

wall (Figure 4). The central region of the outermost part of the epidermal cell is modified into a papilla, but the surface also appears to be foveolate (Figures 3 and 4).

In the case of *E. glandulosum*, the papillae are arranged in rows and have 2 almost foveolate cells between them making this taxon particularly distinctive. At the same time, however, the papilla is a complete cell, not just a modified outer wall (Figures 1 and 7).

The smooth or non-papillate seed coat type is of 2 forms (Table, Figures 5 and 6). One as is exemplified by *E. latifolium* and *E. angustifolium* is only superficially sculptured (Figure 6) whereas the other is foveolate, the centre of the cell being sunken and somewhat alveolate in its sculpturing. In sections (Figures 7-11) these seed



Figs. 7-11. Light microscope sections, $\times 1,125$.
 Fig. 12. E.M. section papilla wall, $\times 24,375$.

surfaces, especially the epidermal cells, are very different. The *latifolium-angustifolium* type has unthickened outer wall (Figure 11), whereas the foveolate types have extremely thickened outer wall, almost to the extent that the epidermal cell contents are excluded (Figure 10).

The distinct differences between these seed coat types indicates a possible genetic discontinuity both within and between species. If the genetic relationship between different seed coat morphologies is a simple one, and there is every reason to think so because of the lack of 'intermediate' forms, then it should be possible to demonstrate biochemical affinities between taxa. Such investigations are underway based on the above findings with respect to the flavonoid and isoenzyme profiles of the taxa investigated.

Résumé. Nous avons examiné au MEB et au microscope ordinaire l'épiderme de la graine de 17 formes du genre *Epilobium* provenant de l'Amérique du Nord et relevé l'utilité et la valeur taxonomique et génétique des données obtenues dans le cadre du genre.

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 16 April 1974.

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